DECEMBER, 1915 BULLETIN 209

VIRGINIA POLYTECHNIC INSTITUTE
VIRGINIA AGRICULTURAL EXPERIMENT STATION

FROG-EYE SPOT ON APPLE LEAF.
Photographed in September.

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The Frog-Eye Leaf Spot of Apples

BY

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BLACKSBURG, MONTGOMERY COUNTY, VIRGINIA

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The Frog-Eye Leaf Spot of Apples.[a]

By C. Harvey Crabill.[b]

Of all the apple foliage diseases in Virginia, none is more prevalent than the frog-eye leaf spot. Its range is unlimited in this State. Annual losses as a result of attacks of this disease are hard to estimate, but probably run into hundreds of thousands of dollars. In orchards where judicious spraying is carried on systematically and thoroughly the destructive effects of frog-eye are so reduced as to become almost negligible. Many fruit growers keep the disease under control, but it is manifest that thousands of them do not, and that the losses resulting are heavy.

Fig. 1.—Frog-Eye Leaf Spot as it appears early in the season.

DESCRIPTION OF THE DISEASE.

1.—Symptoms on the Foliage.

The infections which lead to the development of frog-eye leaf spots begin as soon as the leaves unfold in spring, and continue throughout the summer. The first indications of the disease are apparent about May 1st, when very small circular brown spots, one-sixteenth to one-eighth of an inch in diameter, begin to show up on the foliage. These spots gradually enlarge to about one-eighth to one-fourth of an inch in diameter, maintaining a circular outline and always bordered by a narrow ring of purple. At this point the spots seem to cease growth for a time and become lighter in color. This condition is apparent during the first two weeks in July. (Fig. 1.)

Some of these spots remain in this condition for the remainder of the season. A few of them fall out, leaving a shot-hole effect. The majority of the spots, however,

a. Paper No. 41 from the Laboratories of Plant Pathology and Bacteriology, Virginia Agricultural Experiment Station.

b. Thanks are due to Dr. H. S. Reed, Dr. J. S. Cooley, Mr. J. T. Rogers, Mr. H. E. Thomas and Mr. A. N. Hodgson, who have from time to time worked on this problem and contributed to this paper.

as if suddenly stimulated to further activity, begin a secondary growth. From one or more points on its margin the spot begins to spread, forming brown crescents much darker than the older portion of the spot. These new brown crescents are always bordered with purple on the outer edge. They extend outward and sideways until they finally come together, completing the circle of brown around the gray center. More crescents of brown develop from this first brown circle growing together and forming a second brown circle. This formation of concentric circles continues until the leaves fall in autumn or as a result of the injury, the older rings becoming lighter in color with age. The spot at the time of leaf fall is a more or less irregular blotch made up of a light gray center, sharply defined, surrounded by many concentric rings of brown with lighter zones between. (Frontispiece.)

2.—Effects of the Disease.

When infection is severe many of the leaves turn yellow and fall off by mid-summer. In many instances on record trees have been almost

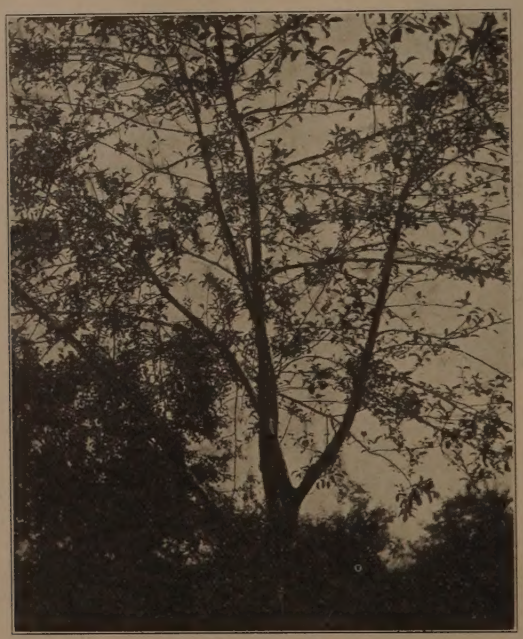

Fig. 2.—A Ben Davis Apple Tree almost defoliated by Frog-Eye Leaf Spot. Photographed August 3.

entirely defoliated. (Fig. 2.) The general effect of this destruction of foliage is to cut down the whole vitality of the tree. As a result the fruit crop for two seasons is practically rendered worthless. The current year's crop is insufficiently nourished and the fruits remain small and insipid while the succeeding crop is materially reduced by the poor development of fruit buds.

Even where defoliation is not severe, comparatively large portions of the leaves may be killed and rendered useless, the tree suffering in consequence. Repeated attacks greatly devitalize the tree; growth becomes slow and fruiting poor.

THE FUNGI ASSOCIATED WITH THE DISEASE.

When frog-eye spots are young, one-sixteenth to one-eighth of an inch in diameter, no fungi are to be found fruiting on the surface. A few weeks later, however, the spores of many species of fungi may be found, especially after the secondary enlargement of the spots begins, notably, *Alternaria mali, Phyllosticta limitata, Phyllosticta pirina,*[a] *Coniothyrium pirinum, Hendersonia sp., Coryneum foliicolum, Pestalozzia sp., Illosporium malifoliorum, Fusarium sp.*

The presence of so many fungi has so complicated the study of this disease that it has been attributed to several of them by as many different writers.

Alwood[1] first described the frog-eye spot and thought it was caused by *Phyllosticta pirina.* Since that time it has been attributed wholly or in part to *Phyllosticta limitata, Coniothyrium pirinum, Coryneum foliicolum, Illosporium malifoliorum, Phoma mali, Alternaria mali,* atmospheric influences, spray injury, and frost.

In 1907 Scott and Rorer[16] first produced frog-eye spots by artificial inoculation with spores of *Sphæropsis malorum.*[b] Since that time this work has been repeated with like success by Brooks and DeMerritt.[3] These experiments proved that the initial infection was due to Sphæropsis but did

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a. The spots may bear either or both of the species of Phyllosticta, **P. limitata** or **P. pirina.** The spores of these two species are indistinguishable. The cultural characters, however, are distinct. The pycnidia of **P. limitata** seldom open on any artificial culture media except those rich in starch. Those of **P. pirina** open readily on almost all media, expelling large pink spore masses. **P. limitata** produces a dark red-brown pigment when grown on Heyden-Nahrstoff agar. **P. pirina** does not.

In 1907 Sheldon[17] discovered a fungus on apple leaf spot in West Virginia, which agreed with Saccardo's **P. pirinia** in all details except color. After submitting dried specimens to Saccardo, Sheldon named this fungus **Coniothyrium pirinum** on account of the dark spores, regarding it as identical with Saccardo's **P. pirina** and published the transfer. There is, however, a fungus almost constantly associated with **Coniothyrium pirinum** on frog-eye spots which agrees exactly with Saccardo's original description of **P. pirina.** The writer therefore feels justified in retaining the name **P. pirina** for this fungus and in regarding **C. pirinum** as a distinct species. After an examination of herbarium specimens in the Department of Agriculture, Scott and Rorer[16] also believed Saccardo's **P. pirina** to be autonomous.

b. Hesler[10] has recently connected **Sphaeropsis malorum** Pk. with the ascomycete **Physalospora cydoniae** Arnaud. The name Sphaeropsis applying to the conidial form will, however, be retained here.

not account for the secondary enlargement and the presence of numerous other fungi. The fact, further, that no Sphæropsis was re-isolated from these spots led many to the belief that the enlargement of frog-eye spots was due to facultative parasites which entered the small spots already killed by Sphæropsis. In 1912 the writer[6] advanced the opinion that Sphæropsis soon died out in the host tissues and that the enlargement was due probably to Phyllosticta.

Roberts[15] has inoculated apple leaves, previously seared in spots by means of a heated rod, with *Alternaria mali*. He obtained some enlargement of the seared spots. These results, together with the fact that Alternaria is almost constantly found on frog-eye spots led him to believe that Alternaria was the cause of the enlargements. His photographs, however, show that the enlargements produced by artificial inoculation with *A. mali* are not at all typical. They are too uniformly spreading to resemble closely the clear cut crescents of the typical frog-eye spot.

EXPERIMENTS TO DETERMINE THE CAUSE OF THE DISEASE.

In attempting to determine definitely the cause of both the initial injury and the secondary enlargement of frog-eye spots the following experiments were carried out.

1.—Isolation Experiments.

Apple leaves infected with frog-eye spot were collected, thoroughly washed and rubbed with tap water and then rinsed in sterile distilled water. A piece of each spot was cut out with sterile scissors, and thrust into the center of a Petri dish of cooled starch agar.[a] Such a culture will be called a thrust culture.

Material for these thrust cultures was selected from (1) round spots, which had not yet begun to put out secondary crescents; (2) the extreme edge of enlargement crescents. The fungi which grew out into the agar from these bits of tissue are shown in Tables I and II.

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a. Prepared as follows:

Water .....900 cc.		Melt up in autoclave
$MgSO_4$ .....0.5 g.		and while still hot
K_2HPO_4 .....1.0 g.		add and shake in,
KCl .....0.5 g.		Water ...100 cc.
$FeSO_4$ .....0.01 g.		Corn starch, 10 g.
$NaNO_3$ .....2.0 g.		Sterilize in auto-
Agar Agar...20.0 g.		clave.

TABLE I.—*The Fungi Which Grew From Small Circular Spots.*

Date	Number of Thrust Cultures	Sphæropsis	Alternaria	Phyllosticta	Coniothyrium
May 3	49	35	9	5	0
June 3	50	45	15	4	1
July 2	21	14	14	6	6
July 13	10	5	9	5	4

It should be explained that none of the fungi involved had fruited on these spots and that usually more than one of them grew from the same spot.

TABLE II.—*The Fungi Which Grew From the Margins of Enlargement Crescents.*

Date	Number of Thrust Cultures	Sphæropsis	Alternaria	Phyllosticta	Coniothyrium
July 8	32*	30	8	10	8
July 13	18**	16	10	8	0
Aug. 16	35	12	22	29	6

Table I shows that Sphæropsis is constantly present in very young spots. Later it is either dead in the round spots or is outgrown in the thrust cultures by the ever present Alternaria. Subsequent experiments indicate that the latter is usually the case although it is not improbable that the Alternaria may in some cases actually kill Sphæropsis in the host tissue. Those round spots which have turned white have invariably yielded *Phyllosticta pirina.*

Table II shows that Sphæropsis is constantly present in the margin of active enlargements. Several pure cultures of this fungus have been obtained by thrust cultures from these margins. (Fig. 3.) It is apparent also that Alternaria follows the Sphæropsis rapidly. By cutting material for thrust cultures from the center or inner crescents of large spots, Alternaria and other fungi are always obtained, often to the exclusion of Sphæropsis.

Up until the middle of July only four fungi, Sphæropsis, Phyllosticta, Coniothyrium and Alternaria, have been found in the spots. After that

* 8 of these were pure Sphaeropsis.
** 3 " " " " "

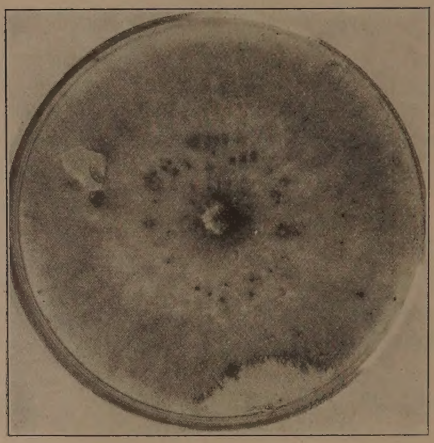

Fig. 3.—A pure culture of Sphæropsis obtained by thrusting a piece of the margin of an enlarged Frog-Eye Spot into starch agar.

date, Pestalozzia, Monochætia, Hendersonia, Coryneum, and Illosporium are more or less frequently encountered. Table III shows the prevalence of these fungi at various parts of the growing season.

TABLE III.—*The Relative Frequency of Different Fungi in Frog-Eye Spot.*

Organism	Occurrence		Earliest Isolation from Spots
	In Very Young Spots	In Enlarged Spots	
Sphæropsis	constant	constant	May 3
Alternaria	frequent	almost constant	May 20
Phyllosticta	"	" "	May 20
Coniothyrium	occasional	frequent	July 5
Pestalozzia	never	infrequent	July 23
Monochætia	"	"	July 25
Hendersonia	"	rare	Nov. —
Coryneum	"	"	Nov. —
Illosporium	"	"	Nov. 15

2.—Inoculation Experiments.

All of the fungi commonly occurring on frog-eye spots have been isolated and studied in pure culture. To determine the role of each of these in connection with the frog-eye spots, several series of inoculations under

controlled conditions have been carried out. Spores from pure cultures were suspended in sterile water and applied to normal healthy leaves, which were bagged as soon as inoculated. Each bag enclosed also a piece of wet cotton. The results of these experiments are presented in Tables IV and V.

TABLE IV.—*Inoculation of Healthy Leaves With Fungi Found Associated with Frog-Eye Spot.*[a]

Date	Fungus	Conditions	Result
June 23, 1915	Sphæropsis malorum	Greenhouse	Positive
July 7, 1915	" "	"	"
June —, 1912	Phyllostieta pirina	Field	Negative
Mar. 26, 1915	" "	Greenhouse	"
June —, 1912	" limitata	Field	"
Mar. 26, 1915	" "	Greenhouse	"
June —, 1912	Coniothyrium pirinum	Field	"
Mar. 25, 1915	" "	Greenhouse	"
July —, 1915	Alternaria mali	Field	"
Aug. —, 1915	" "	Greenhouse	"
June —, 1912	Pestalozzia sp.	Field	"
July 5, 1913	" "	Greenhouse	"
June —, 1912	Monochetia "	Field	"
July 5, 1913	" "	"	"
June —, 1912	Hendersonia "	"	"
July 5, 1913	" "	"	"
July 5, 1913	Coryneum foliicolum	"	"
July 5, 1913	Illosporium malifoliorum	"	"
	Checks	"	"

The spots produced by Sphæropsis in the above experiments were typical of frog-eye spot in every respect. They were first noticed 16 days after inoculation. Fifteen days later enlargements began. From both the young round spots and the enlargements, Sphæropsis were re-isolated in pure state by the thrust culture method. Seventeen such cultures yielded 11 pure Sphæropsis. The other six were contaminated by *Fusarium sp.*

These tests were supplemented by inoculation of leaves in which small holes had been seared with a hot needle.

a. Each set of inoculations was checked by controls which in every case remained healthy.

Fig. 4.—Frog-Eye Leaf Spots as they appear in mid-summer. Some are enlarged, some are whitened and some are just beginning.

TABLE V.—*Inoculation of Seared Leaves.*

Date	Fungus	Condition	Result
June 23, 1915.....	Sphæropsis	Greenhouse	2% typically enlarged
Mar. 26, 1915.....	**P. pirina**	"	No enlargement
Mar. 26, 1915.....	**P. limitata**	"	" "
Mar. 26, 1915.....	**C. pirinum**	"	" "
July —, 1915.....	**A. mali**	Field	" "

Conclusion of Experiments.

As a result of these experiments it can be stated that frog-eye leaf spots, initial infection and enlargements, are caused by *Sphaeropsis malorum*. *Alternaria mali* is a saprophyte spreading through the spot rapidly behind Sphæropsis and rarely becoming a facultative parasite under conditions of excessive moisture. *Phyllosticta pirina*, *Phyllosticta limitata* and *Coniothyrium pirinum* are saprophytes able to grow and fruit on the dead spots but not able to produce enlargement. The light gray color of some round frog-eye spots which have ceased to enlarge is due to infection with *P. pirina*. (Fig. 4.) Coryneum, Hendersonia, Pestalozzia, and Illosporium, which appear on the dead tissue late in the season, are saprophytes.

THE RELATION OF FROG-EYE LEAF SPOT TO BLACK ROT AND CANKER.

The frog-eye spot is caused by *Sphaeropsis malorum*, the same organism as that which produces black rot of apple fruits and cankers on trunks and branches. In order to combat this disease it is necessary to understand the relation between its different forms. The control of one involves the control of the others.

Fig. 5.—The Black Rot of Apples. The small mummy furnished Sphæropsis spores for the infection of the apple below it which is completely rotted and on which Sphæropsis pycnidia are almost ripe. The large apple shows Black Rot spreading from an infection of the calyx. The leaves below these apples were badly infected with Frog-Eye Leaf Spot. Photographed August 6.

Black rot may appear on apples at any age but usually is first noticeable when the fruits are about half grown. Minute purple discolorations are the first indications of the disease. On spreading, these form circular, depressed spots, under which the tissue is brown and practically tasteless. (Fig. 5.) Subsequent spreading consists in the addition of brown concentric rings about the young spot and becomes especially rapid after ripening begins. The brown color of the skin of the diseased portion of the apple has given this disease the name "brown rot" in some localities. It is easily distinguished from "bitter rot" in that it lacks the bitter taste.

Apples affected with black rot usually fall when about half the tissue has been destroyed. On the ground they continue to rot and finally dry up into hard black mummies. (Fig. 5.) Others which remain on the tree do likewise and may even hang on all winter. During the autumn and winter, the Sphæropsis in these mummies begins to produce spores which are mature and ready for dissemination at the beginning of the growing period the next spring.

Black rot cankers are formed on trunks, branches and twigs in much the same way. Frequently twigs killed by blight are infected by Sphæropsis and develop cankers. These cankers produce myriads of spores each spring.

It is these cankers and mummied apples which enable the disease to live through the winter and which furnish the Sphæropsis spores for the infection of apple leaves and the production of frog-eye spot. The indications are that infected leaves wintered on the ground do not constitute an important source of infection for the next year's foliage. Badly infected leaves which had passed the winter under trees in the orchard and in wire baskets out of doors have been thoroughly examined during the winter and spring and only a very few Sphæropsis spores could be found. Unusually severe infections of frog-eye, have, however, been repeatedly traced to cankers, and mummies or dried-up rotten apples that had remained on the tree over winter.

THE CONDITIONS WHICH INFLUENCE INFECTION.

The controlling factors of infection are chiefly three:

1.—Abundance of Infective Material.—Cankered limbs and twigs and mummied apples are the sources of the Sphæropsis spores, the dissemination of which begins when the first warm rains in spring soften the pycnidia and cause the exudation of spores. This process begins usually in March but as far as infection is concerned is of no importance until the apple trees leaf out. The careful pruning out of all dead or cankered wood and the destruction of mummies prior to this time will greatly reduce the amount of infective material.

2.—Abundance of Moisture.—The spores of Sphæropsis are capable of germination only in the presence of moisture. Rain or dew is therefore essential to successful infection. Several season's observations show that heavy rainfall in May and June is always accompanied by severe infection with frog-eye leaf spot, while the reverse is true when spring rainfall is slight. However, there is always enough rain in spring to make this disease a serious consideration.

3.—Susceptibility of Hosts.—No variety of apple is immune to frog-eye but some are more susceptible than others. Of the very susceptible varieties, Ben Davis, Winesap, Arkansas, Baldwin and Jonathan are noteworthy. Early Harvest, Grimes, Yellow Newtown, (Albemarle Pippin), Gano and York Imperial are among the least susceptible. Artificial inoculations on York Imperial leaves show that old leaves are as susceptible as young ones; in fact, they appear to be more so. Old and young leaves were inoculated at the same time and kept under the same conditions, the old leaves developed five times as many spots as the young ones.

When the parasite has gained entrance to the host plant there is nothing which can be done to mitigate or stop the injury which it may cause. The control of this fungus can be made successful only by the prevention of infection.

SPRAYING EXPERIMENTS FOR THE CONTROL OF FROG-EYE SPOT.

During the years 1910 to 1914, inclusive, extensive spraying experiments for the control of frog-eye leaf spot have been conducted in various parts of Virginia. Orchards which were badly diseased in previous years were selected for these tests. From fifty to eighty trees of four varieties, York Imperial, Winesap, Ben Davis and Jonathan, were used each year. Unsprayed trees of each variety were retained as checks.

The sprays tested were:

(1) Lime-sulphur (1½ gallons commercial solution, testing 32° Beaume', to 50 gallons water).

(2) Bordeaux mixture (4-5-50).

(3) Copper-lime-sulphur, (1½-50, plus 2 lbs. copper sulphate).

(4) Iron Bordeaux, (4-5-50, plus 3 lbs. iron sulphate).

Beginning just after the petals had fallen from the apple blossoms these sprays were applied three to five times at intervals of 10 to 21 days. On the basis of three applications, 14 days apart, beginning when the petals had all fallen, the average estimated efficiency of each spray material in all the experiments conducted during the five years is numerically expressed in Table VI.

TABLE VI.—*Relative Efficiency of Spray Material in Controlling Frog-Eye Leaf Spot. Perfect=100 Percent.*

Lime-sulphur	95%
Bordeaux mixture	85%
Iron Bordeaux	85%
Copper-lime-sulphur	85%

In some instances all of these sprays were 100 percent efficient, but ordinary lime-sulphur holds the highest average.

The other sprays, all of which contain copper-sulphate, have under certain weather conditions, produced a type of spray injury which in the early part of the summer is almost indistinguishable from young frog-eye leaf spot. These spots, however, remain small and circular throughout the summer. Saprophytic fungi may be isolated from them but Sphæropsis is absent. The initial injury is without a doubt caused by copper compounds and the dead tissue is later infected by saprophytes, principally Phyllosticta and Alternaria.

The close resemblance of this type of spray injury to the true frog-eye spot may account in part for the low efficiency figures for the copper containing mixtures, but since the injury is similar in its effect on the tree, the figures still represent true comparative values.

In all of the experiments unsprayed check trees lost from 25 to 95 percent of their foliage before September, while adjacent sprayed trees retained full coverings of leaves and matured excellent crops of fruit.

RECOMMENDATIONS FOR THE CONTROL OF THE DISEASE.

The control of frog-eye leaf spot and incidently the control of black rot and Sphæropsis canker, is comparatively simple. It involves (1) the destruction of the sources of infection, viz., cankers and mummies or dried-up diseased apples remaining on the tree over winter, and (2) spraying for the prevention of infection.

1.—Destruction of the Sources of Infection.

Cankers and mummies can best be eradicated in winter when the leaves are off the trees. All dead or cankered wood and mummies should be removed from the trees and burned. This can best be done in the regular course of pruning practice.

2.—Spraying to Prevent Frog-Eye Leaf Spot.

We recommend the same treatments for the prevention of this leaf spot that are used by fruit growers to control apple scab. Spraying with Bordeaux or Lime-sulphur (summer strength), before the bloom opens, supplemented by one or two later treatments at intervals of from ten days to two weeks, will prevent the development of either apple scab or frog-eye leaf spot.

By the use of three or four applications of lime-sulphur at intervals of ten to fourteen days, it is possible to almost entirely eliminate both frog-eye leaf spot and black rot, and to keep them under control from season to season.

SUMMARY.

The frog-eye leaf spot, though usually considered as one of the lesser important apple diseases, is a cause of serious losses in neglected orchards.

The small circular brown spots, which are the first evidence of the disease, appear on the leaves about the first of May. The later development of the spots is more or less irregular, though usually they gradually enlarge as the season advances. When infection is severe many of the leaves turn yellow and fall off by midsummer. The injury to and loss of the foliage results in a reduction of the fruit crop, and tends to lower the vitality of the tree.

For a number of years there has been some uncertainty as to what fungus was responsible for the frog-eye spot. This has been due to the fact that a number of different organisms have been found closely associated in the diseased portion of the leaf.

Investigations have shown that both the initial infection and secondary enlargement of frog-eye spot are due to *Sphaeropsis malorum*. Sphæropsis is constantly present in very young spots; after the spot is a few weeks old the spores of many species of fungi may be found on its surface.

The fungus which causes the frog-eye spot is already well known. In addition to this leaf spot, *Sphaeropsis malorum* is also responsible for a canker that occurs on the trunk and branches of the apple tree, and for black rot of the fruit of apples, pears and quinces.

Spraying experiments, which covered a period of five years, have shown that the regular applications of lime-sulphur spray or Bordeaux mixture as employed by fruit growers to control apple scab and black rot, will also prevent damage by frog-eye leaf spot.

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